CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
IMIDACLOPRID

Chemical Code # 3849, Document Processing Number (DPN) # 51950
SB 950 # N/A
Original date: 5/24/93
Revised date: 3/30/04

I. DATA GAP STATUS

Chronic toxicity, rat: No data gap, no adverse effect (other than for oncogenicity, see below)

Chronic toxicity, dog: No data gap, no adverse effects

Oncogenicity, rat: No data gap, possible adverse effect

Oncogenicity, mouse: No data gap, no adverse effects

Reproduction, rat: No data gap, no adverse effects

Teratology, rat: No data gap, possible adverse effect

Teratology, rabbit: No data gap, no adverse effects

Gene mutation: No data gap, no adverse effects

Chromosome effects: No data gap, possible adverse effect

DNA damage: No data gap, possible adverse effect

Neurotoxicity: No data gap, possible adverse effect

Toxicology one-liners are attached.

All record numbers for the above study types through 209393 (Document No. 51950-0474) were examined.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.

File name: T033004
Revised by Thomas Moore, 3/30/04

These pages contain summaries only. Individual worksheets may identify additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

**** 009,-010,-011; 119472, 119473, 119475**; Chronic Toxicity and Cancerogenicity Studies on Wistar Rats (Administration in Food over 24 Months), (Authors: R. Eiben, G. Kaliner; 831; Rat; Bayer AG, Dept. of Toxicology, D-56 Wuppertal 1, West Germany; Report Nos. 100652, 101931, 102658; 9/6/91; NTN 33893 Technical (94.3% purity); 60 animals/sex/group; Doses: (Study #1)-0, 100, 300, 900 ppm, (Study #2)-0, 1800 ppm; Mortality: (104 wks)-O (M:16/100, F:26/100), 100 (M/F:6/50), 300 (M:6/50, F:10/50), 900 (M:6/50, F:13/50), 1800 (M:5/50, F:10/50); Clinical Observations: no treatment-related signs; weight gain reduced in 1800 ppm group (M: 5%), (F: 11%); Hematology: no treatment effect; Serum Chemistry: increased alkaline phosphatase activity (F, 1800 ppm) at 6, 12, 18 months; Gross Pathology: no treatment-related lesions; Histopathology: (non-neoplastic lesions) increased incidence of mineralized particles in colloid of thyroid gland, (neoplastic) cholangiocellular carcinoma in livers of 2 males (1800 ppm); **possible adverse effect:** cholangiocellular carcinoma; NOEL: 100 ppm, based on the incidence of mineralized particles in the thyroid glands of males in the 300 ppm group; Study **acceptable**. (Moore, 4/7/93)

CHRONIC TOXICITY, DOG

** 013; 119478; "52-Week Oral Toxicity (Feeding) Study with NTN 33893 Technical in the Dog" (Author: Allen, T.R., et al, Research and Consulting Co., Itingen, Switzerland, Lab Project ID 100015, 10/19/89); NTN 33893 Tech. (Batch No. 180587, 94.9% purity); 0, 200, 500 and 1250/2500 ppm in feed (pellet-form) to 4 dogs/sex/dose for 52 weeks; the 1250 ppm dose was increased to 2500 ppm at week 17 due to the lack of apparent toxicity; no animals died during the study; there were no treatment-related effects on clinical signs, body weights, ophthalmoscopy, hearing, hematology or urinalysis; there was a slight but nonsignificant increase of liver weights in both sexes of the high dose group; there was also a slight increase in plasma cholesterol in females and an increase in liver cytochrome P450 in both sexes at the high dose; NOEL (M/F) = 500 ppm (based on increased liver cytochrome P450); **Acceptable** (Patterson, 4/9/93).

ONCOGENICITY, MOUSE

** 014,-015; 119479; 119480; Carcinogenicity Study on B6C3F1 Mice (Administration in the Food for 24 Months), (Author: B. Watta-Gebert); 832; Mouse; Bayer AG, Department of Toxicology, D-56 Wuppertal 1, West Germany; Study Nos. 100693, 101929; 1/28/91, 10/24/91; NTN 33893 Technical (purity: 95.3%); 60 animals/sex/group; Doses: (Study #1) 0, 100, 330, 1000 ppm, (Study #2) 0, 2000 ppm; Mortality: 0 (M:9/100, F:26/100), 100 (M:6/50, F:7/50), 330 (M:3/50, F:9/50), 1000 (M:8/50, F:9/50), 2000 (M:17/50, F:14/50); Clinical Observations: no treatment-related effects; weight gain reduced (2000 ppm) (M: 29%, F: 26%); Hematology: reduced wbc count (2000 ppm) (1000 ppm, F only); Serum Chemistry: alk. phosphatase activity increased (2000 ppm), cholesterol level decreased (2000 ppm), urea level decreased (2000 ppm, M only); Gross Pathology: no treatment-related lesions; absolute liver, brain, lung, spleen, kidney, and adrenal gland (F only) weights decreased (2000 ppm), liver (F only, 1000 ppm); relative liver, spleen weights decreased (F only, 2000 ppm); Histopathology: slight periacinar hepatocytic hypertrophy (M only, 2000 ppm); mineralization in the thalamus (F only, 2000 ppm);

no treatment-related incidence of neoplasms; **no adverse effect identified;** NOEL: 1000 ppm, (estimated compound intake: 143.1 mg/kg/day), based on reduced weight gain and increased mortality of animals in 2000 ppm group; Study acceptable. (Moore, 4/9/93)

016; 119481; Pilot Range-Finding Study for a Cancerogenesis Study on B6C3F1 Mice, (Author: R. Eiben); 821; Mouse; Bayer AG, Department of Toxicology, D-56 Wuppertal 1, West Germany; Report No. 99808; 10/24/88; NTN 33983 Technical (purity: 92.8%); 10 animals/sex/group; Doses: 0, 120, 600, 3000 ppm; Mortality: 0 (M/F:0/10), 120 (M:1/10, F:0/10), 600 (M:1/10, F:0/10), 3000 (M/F:7/10); Clinical Observations: poor appearance (3000 ppm), significant reduction of body weight gain (3000 ppm); Hematology: no treatment-related effects; Serum Chemistry: elevation of alkaline phosphatase (3000 ppm); Gross pathology: no treatment-related lesions, reduced absolute brain, heart, liver, kidneys, spleen (F only), and adrenals (F only) weights (3000 ppm), reduced relative liver (F only), heart, and spleen (F only) weights (3000 ppm); Histopathology: no treatment-related lesions; target organ not identified; no possible adverse effect identified; NOEL: 600 ppm, (estimated daily intake: 85.7 mg/kg/day), based on poor appearance, reduced weight gain and increased mortality in the 3000 ppm group; Study **supplemental**. (Moore, 4/8/93)

REPRODUCTION, RAT

** 019; 119496; Multiple Generation Reproduction Study in Rats, (Authors: P. Suter ~7et. al.~1); RCC, Research and Consulting Company AG, Itingen, Switzerland; Study No. 100647; 6/21/90; NTN 33893 Technical (purity: 95.3%); P generation: 30 animals/sex/group, F1B generation: 26 animals/sex/group; 2 litters/generation; Dose: 0, 100, 250, 700 ppm; Mortality: (P) 0 (M:0/30, F:2/30), 100 (M:0/30, F:1/30), 250 (M/F:0/30), 700 (M/F:0/30), (F1B) 0 (M/F:0/30), 100 (M/F:0/30), 250 (M:1/30, F:0/30), 700 (M/F:0/30); Clinical observations: decreased body weight gain (F0-700 ppm M, F1B F); Hematology: no treatment-related effects; Clinical Biochemistry: increased O-demethylase activity (F1B-250 ppm F, 700 ppm M,F), N-demethylase activity (F1B-700 ppm M), and cyt. P450 activity (F1B-700 ppm, M); Necropsy: no treatment-related lesions, no effect on organ weights; Histopathology: no treatment-related lesions; Reproductive factors: no treatment-related effects on fertility index, litter size; Developmental factors: no treatment-related abnormalities, decreased weight gain (F1A, F1B, F2A, F2B-M,F, 700 ppm), no treatment-related effect on gestation index, viability index, or lactation index; **no adverse effects identified**; NOEL: (parental) 700 ppm, (reproductive) 700 ppm, (developmental) 250 ppm (based on decreased weight gain for pups, 700 ppm); **Study acceptable.** (Moore, 4/14/93)

TERATOLOGY, RAT

**** 017; 119482**; Embryotoxicity Study (including Teratogenicity) with NTN 33893 Technical in the Rat (Authors: H. Becker, ~7et. al.~1); 833; Rat; RCC, Research & Consulting Company AG, Itingen, Switzerland; Study No. 98571; 1/8/92; NTN 33893 Technical (purity: 94.2%); 25 females/group; Doses 0, 8.9, 25.9, 94.1 mg/kg/day (analytical), test material administered by gavage from day 6 post coitum through day 15; No mortality; Clinical observations: no treatment-related signs, mean food consumption and body weight gain decreased during treatment period (94.1 mg/kg/day); Necropsy: no treatment-related lesions; Developmental: high percentage of male fetuses, increased incidence of wavy ribs (94.1 mg/kg/day); **possible adverse effect:** increased percentage of male fetuses; **Maternal NOEL =** 25.9 mg/kg/day (based on

decreased body weight gain and reduced food consumption of the 94.1 mg/kg/day treatment group; **Developmental NOEL =** 25.9 mg/kg/day (based on increased incidence of wavy ribs in the fetuses of the 94.1 mg/kg/day treatment group); Study **acceptable**. (Moore, 4/19/93)

TERATOLOGY, RABBIT

** 018; 119484; Embryotoxicity Study (including Teratogenicity) with NTN 33893 Technical in the Rabbit, (Authors: H. Becker, K. Biederman); 833; Rabbit; RCC, Research and Consulting Company AG, CH 4452 Itingen, Switzerland; Study No. 98572; 1/8/92; 16 females/group; Doses: 0, 7.0, 20.5, 64.3 mg/kg/day (analytical), doses administered by gavage from day 6 post coitum through day 18; Mortality: 0 (0/16), 7.0 (0/16), 20.5 (0/16), 64.3 (2/16); Clinical observations: reduced food consumption, body weight loss day 6 to 19, one abortion (64.3 mg/kg/day), reduced body weight gain day 6 to 19 (20.5 mg/kg/day); Necropsy: no treatment-related lesions; Developmental: one abortion, two total resorptions, increased post-implantation loss, reduced mean fetal weight (64.3 mg/kg/day); **no adverse effects**; **Maternal NOEL =** 20.5 mg/kg/day (based on mortality of dams, decreased body weight gain for 64.3 mg/kg/day treatment group); **Developmental NOEL =** 20.5 mg/kg/day (based on increased post-implantation loss, decreased fetal weight of the offspring in the 64.3 mg/kg/day treatment group); Study **acceptable**. (Moore, 4/16/93).

GENE MUTATION

51950-020; 119497; mutagenicity; 842; "NTN 33893; Reverse Mutation Assay (~7Salmonella typhimurium and Escherichia coli~1)"; author, M. Watanabe; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Hino Institute, Toxicological Research Laboratory, Japan; 1/17/91; report #101276; Imidacloprid Technical (NTN 33893; 93.7% purity); doses (+/- S9 microsomes): 312.5, 625, 1250, 2500, & 5000 mg/plate, triplicate cultures, 2 independent trials; ~7S. typhimurium~1 tester strains TA98, 100, 1535, & 1537 and ~7E. coli~1 strain WP2/uvrA; positive controls +/- S9 were successful in all instances; 48 hr exposure; **no adverse effects: there was no evidence for mutagenicity (~7i.e.~1 an increase in revertants arising in low-histidine or low typtophan medium) in any tester strain, regardless of the presence or absence S9 activating microsomes; **Acceptable**. (Rubin, 4/8/93)

51950-020; 119498; mutagenicity; 842; "NTN 33893; Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay" author, H. Lehn; Bayer AG, Institute of Toxicology, FRG; 1/6/89; report #98584; Imidacloprid Technical (NTN 33893; 95.2% purity); doses ranged between 0-125 mg/ml in the absence of S9 activating microsomes and between 0-1222 mg/ml in the presence of S9; 5 hr exposure; cytotoxicity was evident directly after treatment with 70 and 80 mg/ml test article, +/- S9, respectively; **no adverse effects: no increase in 6-thioguanine resistance was measured under any condition, thus NTN 33893 is not considered mutagenic in this system; **Acceptable**. (Rubin, 4/8/93)

** 51950-020; 119499; mutagenicity; 842; "NTN 33893; Salmonella/Microsome Test to Evaluate for Point Mutagenic Effects"; Bayer AG, Institute of Toxicology, FRG; author, B.A. Herbold; 1/6/89; report #98570; Imidacloprid Technical (NTN 33893; 95.0% purity); ~7S. typhimurium~1 strains TA 98, TA 100, TA 1535, & TA 1537; doses, Test #1 (+/- 30% S9): 0, 20, 100, 500, 2500, & 12,500 mg/plate; Test #2 (-S9, +10% S9, & +30% S9): 0, 775, 1550, 3100, 6200, & 12,400 mg/plate; slight cytotoxicity at high dose based on titer determinations in

high-histidine agar; **no adverse effects:** no evidence for mutagenicity (~7i.e.~1 an increase in revertants arising in low-histidine agar) in any tester strain, regardless of the presence or absence S9 microsomes and despite the success of the positive control compounds; **Acceptable**. (Rubin, 4/14/93)

CHROMOSOME EFFECTS

** 51950-020; 119500; structural chromosome aberration; 843; Chinese hamsters; "NTN 33893; In Vivo Cytogenetic Study of the Bone Marrow in Chinese Hamster to Evaluate for Induced Clastogenic Effects"; Bayer AG, Institute of Toxicology, FRG; author, B.A. Herbold; 11/24/89; report #100021; Imidacloprid Technical (NTN 33893; 94.6% purity); dose: 2000 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 30 mg/kg cyclophosphamide; animals sacrificed at 6, 24, & 48 hr post dose (positive & negative controls were sacrificed at 24 hr only); 5/sex/sacrifice group; deaths: 4/34 animals treated with test article from acute toxicity; no variations of biological significance were seen in chromosomal integrity among all treatment groups and negative controls; positive controls exhibited large increases in % metaphases with aberrations; **no adverse effects:** NTN 33893 is not clastogenic in this assay under the conditions tested; **Acceptable**. (Rubin, 4/15/93)

**** 51950-020**; 119501; structural chromosome aberration; 843; "NTN 33893; In Vitro Cytogenetic Study with Human Lymphocytes for the Detection of Induced Clastogenic Effects"; Bayer AG, Institute of Toxicology, FRG; author, B.A. Herbold; 6/16/89; report #99262; Imidacloprid Technical (NTN 33893; 95.2% and 99.8% purity, 1st & 2nd expts., respectively); cells freshly isolated from 1 male & 1 female volunteer; doses (-/+ S9 microsomes), Expt. #1: 0, 50, 500, & 5000 mg/ml; Expt. #2: 0, 1300, 2600, & 5200 mg/ml; cytotoxicity, indicated by a decline in mitotic index, was most prominent w/o S9 (declines to 64% of control @ 500 mg/ml in Expt. #1 and 41.4% of control @ 1300 mg/ml in Expt. #2) and was only weakly apparent w/S9; clastogenesis, indicated mainly by the appearance of chromosomal gaps & breaks, was also most prominent w/o S9 (metaphases w/aberrations excluding gaps increased from 3.0% in controls to 14% @ 5000 mg/ml w/no effect @ 50 & 500 mg/ml in Expt. #1 and from 2.0% to 10.0 and 28.0% @ 1300 & 2600 mg/ml in Expt. #2); only weak clastogenic effects seen in the presence of S9; **possible adverse effects:** NTN 33893 is clastogenic in this assay under the conditions tested; **Acceptable**. (Rubin, 4/16/93)

** 51950-020; 119503; structural chromosome aberration; 843; Mouse; "NTN 33893; Micronucleus Test on the Mouse to Evaluate for Clastogenic Effects"; author, B.A. Herbold; Bayer AG, Institute of Toxicology, FRG; 6/27/88; report #102652; Imidacloprid Technical (NTN 33893; 95.3% purity); dose: 80 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 20 mg/kg cyclophosphamide; animals sacrificed 24, 48, & 72 hr post dose; 5/sex/sacrifice group; **no adverse effects:** no test article-induced statistically significant increase over negative controls was observed in the number of micronucleated polychromatic or normochromatic cells despite the success of the positive controls; no statistically significant alterations occurred in the ratio of polychromatic to normochromatic cells; **Acceptable**. (Rubin, 4/19/93)

** 51950-020; 119504; structural chromosome aberration; 843; Mouse; "Mouse Germ-Cell Cytogenetic Assay with NTN 33893"; author, W. Volkner; Cytotest Cell Research GmbH & Co. KG, In den Leppsteinswiesen 19, Robdorf, FRG; 5/22/90; report #102654; Imidacloprid

Technical (NTN 33893; 94.1% purity); dose: 80 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 10 mg/kg doxorubicin sulfate HCl dosed in saline; animals sacrificed 6, 24, & 48 hr post dose; 6/males/sacrifice group (only 5 were evaluated); spermatogonia were isolated from both testes and prepared on slides; despite a successful positive control, the test article failed to induce any biologically relevant increase in spermatogonial chromosome aberrations; neither the positive control nor the test article had an effect on mitotic index; **no adverse effects:** under the conditions tested, NTN 33893 is neither clastogenic nor cytotoxic to mouse spermatogonia; **Acceptable**. (Rubin 4/20/93)

DNA DAMAGE

51950-020/158; 119502/128284; other genetic effects; 844; Chinese hamsters; "NTN 33893; Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters In Vivo"; author, B.A. Herbold; 6/16/89 (original), 11/11/93 (supplement); Report #99257-1; Imidacloprid Technical (NTN 33893; 95.0% purity); doses: 0, 500, 1000, & 2000 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg b.w.); positive control: 10 mg/kg cyclophosphamide (CP); animals sacrificed 24 hr post dose, 2 hr after colcemid treatment to arrest cells in metaphase; 5/sex/dose (50 metaphases/animal analyzed for SCE); marrow preparations made from the femur; no deaths; no toxic clinical signs; cytotoxicity was present at 1000 and 2000 mg/kg (mitotic index declined at both doses to 83.3% of controls); no change in proportion of cells in 1st, 2nd, & 3rd metaphases indicating no effect on cell cycling; sister chromatid exchange rate was also unaffected (SCE mean rate per metaphase was 2.01, 2.17, 2.28, & 2.41 for 0, 500, 1000, & 2000 mg/kg, respectively) despite successful positive control (SCE rate was 15.27 for 10 mg/kg CP, $p<.01$); Acceptable. (Rubin, 4/19/93; revised from unacceptable with submission of individual animal data by Rubin, 3/8/94)

**** 51950-020**; 119505; other genetic effects; 844; "Clastogenic Evaluation of NTN 33893 in an In Vitro Cytogenetic Assay Measuring Sister Chromatid Exchange in Chinese Hamster Ovary (CHO) Cells"; author, R.D.F.M. Taalman; Hazleton Biotechnologies, Landjuweel, Veenendaal, The Netherlands; 4/21/88; report #102655; Imidacloprid Technical (NTN 33893); 95.2% purity; doses, -S9, Trial I: 16.7, 50, 166.7, & 500 mg/ml; Trial II: 100, 250, 500, & 1000 mg/ml; +S9, Trial I: 166.7 & 500 mg/ml and 1.7 & 5.0 mg/ml; Trial III: 500 mg/ml and 1, 2, & 3 mg/ml; Trial II/-S9 and Trial III/+S9 gave results indicating a dose-dependent rise in SCE/diploid cell (4, 44, 56, & 96% over solvent control for Trial II/-S9 and 0, 8, 28, & 70% over solvent control for Trial III/+S9); cytotoxicity was present at concentrations above (and including) 500 mg/ml -S9 and at 3 mg/ml +S9; **possible adverse effects:** NTN 33893 induces SCE in CHO cells in the absence and presence of S9 under the conditions tested; **Acceptable**. (Rubin 4/21/93)

** 51950-020; 119506; other genetic effects; 844; "Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells"; author, D.L. Putnam & M.J. Morris; Microbiological Associates, Inc., Rockville, MD; 9/12/89; report #99676; Imidacloprid Technical (NTN 33893); 95.2% purity; doses, -S9: 25, 50, 100, 200, & 400 mg/ml; +S9: 157, 313, 625, & 1250 mg/ml; **no adverse effects:** no evidence for induction of SCE in the presence or absence S9 in this system despite cytotoxicity present at each dose tested; **Acceptable**. (Rubin 4/21/93)

** 51950-020; 119507; other genetic effects; 844; ~7Bacillus subtilis~1; "NTN 33893; Rec-assay with Spores in the Bacterial System"; author, M. Watanabe; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Hino Institute, Toxicological Research Laboratory, Japan;

6/18/90; report #101275; Imidacloprid Technical (NTN 33893; 94.7% purity; doses (-/+ S9): 312.5, 625, 1250, 2500, 5000 mg/disk; positive controls, mitomycin C (-S9) and 2-aminoanthracene (+S9) successfully generated large differences in growth inhibition zone between the Rec+ and Rec- ~7B. subtilis~1 strains H17 and M45, respectively, indicating a positive gene damaging effect; **no adverse effects:** no test article-induced differences were observed in growth inhibition zones between the 2 strains, thus no damage occurred which a Rec+ DNA repair system might have remedied; **Acceptable**. (Rubin 4/22/93)

** 51950-020; 119508; other genetic effects; 844; "Mutagenicity test on NTN 33893 in the Rat Primary Hepatocyte Unscheduled DNA Synthesis [UDS] Assay"; (author, M.A. Cifone; Hazleton Laboratories America, Inc., Kensington, MD, report #98573, 12/21/88); Imidacloprid Technical (NTN 33893); 95.2% purity; 5 trials, cells isolated from each of 2 rats/trial; Trials 1, 2, & 4 were non-functional; doses, Trial 3: 5 (Rat #2 only), 10, 25, 50, 100, 250, & 500 mg/ml; doses, Trial 5: 50, 100, 250, 375, 500, 750 mg/ml; higher concentrations not analyzed because of excessive toxicity; UDS assessed by autoradiographic determination of 3H-thymidine incorporation; criteria for positive UDS response (net nuclear grain count more than 6 above negative controls, % nuclei w/$\underline{\geq}$ 6 grains was at least 10% of the population more than controls, % nuclei w$\underline{\geq}$ 20 grains exceeds 2% of the population) not fulfilled at any dose (the positive control, 2-acetyl aminofluorene, was successful); however; there was evidence for a weakly positive response at high doses; **no adverse effects**; **Acceptable**. (Rubin 4/23/93)

** 51950-020; 119509; other genetic effects; 844; "NTN 33893; Test on ~7S. cerevisiae D7~1 to Evaluate for Induction of Mitotic Recombination"; author, B.A. Herbold; Bayer AG, Institute of Toxicology, FRG; 6/27/88; report #102653; Imidacloprid Technical (NTN 33893); 95.3% purity; 2 trials; single test tube/dose replated onto 10 plates in complete agar medium to detect mitotic crossing over by colony color or in tryptophan-deficient agar to detect mitotic gene conversion; doses: 0, 625, 1250, 2500, 5000, 10000 mg/ml; positive controls: -S9, methyl methane sulfonate; +S9, cyclophosphamide; **no adverse effects:** since there were no changes in the numbers of red or pink colonies or in the ability to grow in tryptophan-deficient medium as compared to negative controls, there was no evidence of the occurrence of recombination events, either in the form of crossing over or gene conversion; positive controls stimulated both types of recombination; **Acceptable**. (Rubin 4/26/93)

ACUTE NEUROTOXICITY

51950-0472, -0473; 209391, 209392; “An Acute Oral Neurotoxicity Screening Study with Technical Grade Imidacloprid (NTN 33983) in Rats”; (L.P. Sheets; Miles Inc., Agriculture Division, Toxicology, Stilwell, KS; Study Nos. 106348, 106348-1; 2/16/94 and 6/7/94); Two acute neurotoxicity studies were performed. In the 1st study, eighteen Sprague-Dawley rats/sex/group were dosed orally by gavage with 0, 42, 151 or 307 mg/kg of Imidacloprid Technical (NTN 33893 technical, batch no. 2030030, purity: 98.8% (8/92)). Six animals/sex/group were identified as the satellite animals and used for clinical pathology testing. In the 2nd study, 12 females/group were likewise dosed orally with 0 or 20 mg/kg of the test material (same batch no., purity: 98.6% (4/94)). In the 1st study, 4 males and 10 females in the 307 mg/kg group died within two days of dosing. In the functional observational battery (FOB) performed 90 minutes after dosing, some of the 307 mg/kg group animals displayed tremors and incoordination in their gait in the home cage and open field tests. In the home cage, some of these animals exhibited greater or less than normal activity levels. In the open field test, the

animals were generally more sluggish in their movements. The mean frequency of rearing was also reduced for both sexes of this group (M: NS, F: <0.05). In the reflex/physiologic testing, some of the animals in the high dose had no reaction to touch, auditory or pinch stimuli. For the 151 mg/kg group females, one of the 12 animals exhibited tremors in the FOB on Day 0. Mean hindlimb strength was lower for the 307 mg/kg males on Day 0. Mean motor and locomotor activities for both sexes in the 151 and 307 mg/kg groups were lower than those of the control on Day 0. Although some of the values for the hematological and clinical chemical parameters in the 307 mg/kg group were significantly different from those of the control, these differences were not considered to be toxicologically relevant. In the necropsy examination, the mean absolute brain weight for the 307 mg/kg males was less than that of the control ($p<0.05$), the relative weights were not significantly different. No treatment-related effects were noted in the 2nd study. **Possible adverse effect:** tremors and other signs of neurotoxicity; **NOEL (M/F):** 42 mg/kg (based upon the decreased motor and locomotor activity levels and presence of tremors in the 151 mg/kg treatment group); **Study acceptable.** (Moore, 3/3/04)

SUBCHRONIC NEUROTOXICITY

51950-0471; 209390; "A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade Imidacloprid (NTN 33983) in Fischer 344 Rats"; (L.P. Sheets; Miles Inc., Agriculture Division, Toxicology, Stilwell, KS; Study No. 106356; 6/13/94); Eighteen Fischer 344 rats/sex/group received 0, 140, 963 or 3027 ppm of Imidacloprid Technical (NTN 33893 technical, batch no. 2030030, purity: 97.6% (3/93)) in the diet for 13 weeks ((M) 0, 9.3, 63.3, 196 mg/kg/day, (F) 0, 10.5, 69.3, 213 mg/kg/day). Six animals/sex/group were used as satellite animals of use in the hematology and clinical chemistry evaluations. No deaths occurred during the study. The mean body weights and food consumption of both sexes in the 963 and 3027 ppm groups were lower than those of the control group ($p<0.05$). In the Functional Observational Battery (FOB), although the mean hindlimb grip strength of the 3027 ppm males was lower after 8 weeks of treatment ($p<0.05$) and a greater number of these males had a slightly uncoordinated righting reflex at 13 weeks ($p<0.05$), these results did not indicate a consistent effect and were considered to be incidental. Otherwise, no other effects were evident in the FOB. In the clinical chemistry evaluation, serum triglyceride concentrations were lower for both sexes in the 3027 ppm group at both 4 and 13 weeks ($p<0.05$). Mean phosphate levels were reduced for both sexes in the 3027 ppm group at 4 weeks and for the males in that group at 13 weeks ($p<0.05$). The mean albumin concentrations for the 3027 ppm females were lower than those of the controls at both 4 and 13 weeks ($p<0.05$). Although the mean values of other parameters for the 3027 ppm group demonstrated an increase or decrease over the values for the controls, the observed effects were not consistent over the course of the study or were doubtful toxicological significance. There were no treatment-related effects evident in the hematology results, the necropsy or the histopathology examinations. No signs of neurotoxicity were noted. **No adverse effect indicated. Subchronic NOEL (M/F):** 140 ppm ((M) 9.3 mg/kg/day, (F) 10.6 mg/kg/day) (based upon lower mean body and food consumption of the 963 ppm group). **Study acceptable.** (Moore, 3/5/04)

DEVELOPMENTAL NEUROTOXICITY

51950-0474 209393 Sheets, L. P., "A developmental neurotoxicity screening study with technical grade Imidacloprid in Wistar Rats," Bayer Corp., Stilwell, KS, 9/14/01. Study # 99-D72-DV: Bayer Report No. 110245. Thirty Crl:W(HAN)BR mated females/group were dosed in diet with 0, 100, 250, or 750 ppm imidacloprid (98.2% purity) throughout gestation and lactation (ending lactation day 21). Estimated mean gestation exposures were 8.2, 19, and 57 mg/kg/day. Estimated mean exposures during lactation days 0-14 were 0, 15, 36, and 104 mg/kg/day. At least 21 litters per group were of sufficient size to maintain offspring until sacrifice at about postnatal day (PND) 75. Maternal NOEL = 250 ppm, based on transient reduction in food consumption during lactation days 0-7. Developmental toxicity NOEL cannot be determined because intermediate groups were not evaluated in the presence of a conspicuous change in 750 ppm in morphometric measurements (see below). Most endpoints other than the morphometric measurements were evaluated in intermediate dose levels, and none of these found treatment effects at 250 ppm. Findings at 750 ppm in offspring were reduced mean pup weight (5 g) at PND 21 weaning, reduced motor activity in PND 17 males and females and in PND 21 females, modest reductions in motor and locomotor activities during the first recording interval in PND 60 males (suggesting a slight reduction in exploratory activity in a novel environment), and a substantial reduction in the thickness of the corpus callosum in PND 11 females only (not reflected in PND 75 rats of either sex). Study is not acceptable, and appears not to be upgradeable. The apparent corpus callosum change in 750 ppm females at PND 11 indicates a need to analyze intermediate groups. The statistic procedures for PND 11 morphometric measurements need to examined. Morphometric measurements should be performed in intermediate groups wherever an effect is statistically significant at 750 ppm. Cited positive control method validation studies contemporary with this study are requested. See discussion of DPR review for details on concerns about study conduct and report presentation. Other than these issues, this study addressed the full scope of evaluations that pertain to developmental neurotoxicity studies. Aldous, 3/24/04.

STUDIES ON METABOLITES

51950-025; 119521; 842; mutagenicity; "WAK 3839; Reverse Mutation Assay (~7Salmonella typhimurium~1 and ~7Escherichia coli~1)"; author: M. Watanabe; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Hino Institute, Tox. Research Lab., Japan; 11/26/90; report #100668; WAK 3839, a metabolite of NTN 33893; 98.3% purity; ~7S. typhimurium~1 strains TA98, TA100, TA1535, & TA1537, and ~7E. coli~1 strain WP2/uvrA; doses (-/+ S9): 312.5, 625, 1250, 2500, & 5000 mg/plate; positive controls were successful; either no effect or very weak effects of test article on revertant frequency were observed; WAK 3839 is not mutagenic in these systems under the conditions tested; **Acceptable**. (Rubin, 4/26/93)

51950-025; 119522; 842; mutagenicity; "WAK 3839; Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HGPRT Assay In Vitro"; author: H. Lehn; Bayer AG, Department of Toxicology, Wuppertal, FRG; 8/15/89; report #100662; WAK 3839, a metabolite of NTN 33893; 98.9% purity; doses (based on solubility limit and cytotoxicity test): 500, 1000, 1500, 1750, & 2000 mg/ml for both -S9 trials and 1 of 2 +S9 trials; for the other +S9 trial the doses were 500, 750, 1000, 1250, 1500, & 1750 mg/ml; after plating 4 x 106 cells/250 ml flask, the cells were exposed to test article (-/+ S9 microsomes) for 5 hr followed by an "expression period" of exponential growth and subsequent replating under selective conditions (10 mg/ml 6-thioguanine) at 3 x 105 cells/100 mm dish; after 7 days the colonies were fixed and counted; duplicate exposure dishes were run, each dish generating 8 replicate dishes in the selection

condition; test article did not induce 6-thioguanine resistance at any dose despite success of positive controls (-S9, ethyl methane sulfonate; +S9, DMBA); it is not mutagenic in this system under these conditions; **Acceptable**. (Rubin, 4/26/93)

51950-025; 119523; 842; mutagenicity; "WAK 3839; Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay In Vitro"; author: H. Lehn; Bayer AG, Department of Toxicology, Wuppertal, FRG; 2/22/89; report #100661; WAK 3839, a metabolite of NTN 33893; 94.3% purity; doses (based on solubility limit and cytotoxicity test), -S9: 62.5, 125, 250, 500, 1000, & 2000 mg/ml; +S9: 500, 750, 1000, 1250, 1500, & 2000; after plating 4 x 106 cells/250 ml flask, the cells were exposed to test article (-/+ S9 microsomes) for 5 hr followed by an "expression period" of exponential growth and subsequent replating under selective conditions (10 mg/ml 6-thioguanine) at 3 x 105 cells/100 mm dish; after 7 days the colonies were fixed and counted; duplicate exposure dishes were run, each dish generating 8 replicate dishes in the selection condition; test article did not consistently induce 6-thioguanine resistance at any dose despite success of positive controls (-S9, ethyl methane sulfonate; +S9, DMBA); it is not mutagenic in this system under these conditions; **Acceptable**. (Rubin, 4/27/93)

51950-025; 119524; 843; structural chromosome aberration; "WAK 3839 or NTN37571; Micronucleus Test on the Mouse After Intraperitoneal Injection"; author: B.A. Herbold; Bayer AG, Department of Toxicology, Wuppertal, FRG; 10/3/89; report #100664; WAK 3839 (aka NTN 37571), a metabolite of NTN 33893; 98.9% purity; dose (based on pilot toxicity test): 0 & 50 mg/kg body wt., administered intraperitoneally as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 20 mg/kg cyclophosphamide (sacrificed @ 24 hr only); animals sacrificed 24, 48, & 72 hr post dose, bone marrow erythroblasts isolated from femur; 5/sex per sacrifice group; no test article-induced increase over negative controls was observed in the # of micronucleated polychromatic or normochromatic cells despite the success of the positive controls; **Acceptable**. (Rubin, 4/27/93)

51950-025; 119525; 843; structural chromosome aberration; "NTN 37571: Micronucleus Test on the [sic] Mice After I.P. Treatment; Pilot Study"; author: M. Usami; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Agricultural Chemicals Institute, Japan; 11/29/88; report #100679; NTN 37571 (aka WAK 3839), a metabolite of NTN 33893; 96.4% purity; doses: 0, 20, 40, & 80 mg/kg body wt., administered intraperitoneally as a suspension in DMSO:olive oil (1:10, 10 ml/kg); positive control: 4 mg/kg mitomycin C; animals sacrificed 30 hr post dose, bone marrow erythroblasts isolated from femur; 5 males/dose; no test article-induced increase over negative controls was observed in the # of micronucleated polychromatic or normochromatic cells despite the success of the positive controls; no change in the polychromatic/normochromatic cell ratio; **Unacceptable** (no females were tested, only a single sampling time was tested, and no individual data were presented). (Rubin, 4/27/93)

51950-025; 119527; 843; structural chromosome aberration; "WAK 3839; Micronucleus Test on the Mouse After Oral Application"; author: B.A. Herbold; Bayer AG, Department of Toxicology, Wuppertal, FRG; 10/3/89; report #100663; WAK 3839, a metabolite of NTN 33893; 98.9% purity; dose (based on pilot toxicity test): 100 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 20 mg/kg cyclophosphamide; animals sacrificed 24, 48, & 72 hr post dose, bone marrow erythroblasts isolated from femur; 5/sex per sacrifice group; the 48-hr sacrifice group showed a statistically significant increase over controls in micronucleated polychromatics (2.0/1000 % 0.8 ~7vs~1

0.7/1000 % 0.9 in controls sacrificed at 24 hr, p<0.01) which may be partially accounted for by the abnormally low value of the controls compared to historical controls; slight non-statistically significant increases over negative controls were also observed in the # of micronucleated polychromatic cells in the 24- and 72-hr sacrifice groups; positive controls sacrificed at 24 hr raised the # of micronucleated cells to 16.1 % 7.9 per 1000 polychromatics; there may be a weak effect of the test article on micronucleus formation under these conditions; **Acceptable**. (Rubin, 4/27/93)

51950-025; 119528; 843; structural chromosome aberration; "NTN 37571: Micronucleus Test on the [sic] Mice After Oral Treatment; Pilot Study"; author: M. Usami; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Agricultural Chemicals Institute, Japan; 11/29/88; report #100680; NTN 37571 (aka WAK 3839), a metabolite of NTN 33893; 96.4% purity; doses (based on a preliminary toxicity determination): 0, 40, 80, & 160 mg/kg body wt., administered by gavage as a suspension in DMSO:polyethylene glycol 400 (1:5, 10 ml/kg); positive control: 4 mg/kg mitomycin C, injected intraperitoneally; animals sacrificed 30 hr post dose, bone marrow erythroblasts isolated from femur; 5 males/dose; no test article-induced increase over negative controls was observed in the # of micronucleated polychromatics or change in the polychromatic/normochromatic cell ratio despite the success of the positive controls in raising the # of micronucleated polychromatics and lowering the polychromatic/normochromatic ratio; **Unacceptable** (no females were tested, positive controls were not administered by the same route as the test article, only a single sampling time was used, and no individual data were provided). (Rubin, 4/28/93)

51950-025; 119529; 843; structural chromosome aberration; "Chromosome Aberration Assay in Chinese Hamster V79 Cells In Vitro with WAK 3839"; author: A. Heidemann; Cytotest Cell Research GmbH & Co., Robdorf, FRG; 9/27/89; report #100666; 98.8% purity; doses (based on a preliminary cytotoxicity determination and test article solubility), -/+ S9: 0.1, 0.3, & 1.0 mg/ml; cultures harvested 7 (high dose only), 18, & 28 (high dose only) hr after start of the 4 hr exposure; positive controls ethyl methane sulfonate (-S9) and cyclophosphamide (+S9) showed distinct increases in aberrations; despite cytotoxicity of the test article at the mid and high dose indicated by a decline in mitotic index and at the high dose by a decline in plating efficiency (-S9 only), there was no increase in chromosome aberrations; WAK 3839 is not clastogenic in this system under these conditions; **Acceptable**. (Rubin, 4/28/93)

51950-025; 119530; 843; structural chromosome aberration; "NTN 37571: In Vitro Cytogenetic Assay Measuring Chromosome Aberrations in CHO-K1 Cells"; author: M. Usami; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Agricultural Chemicals Institute, Japan; 11/5/88; report #100678; NTN 37571 (aka WAK 3839), a metabolite of NTN 33893; purity not reported; doses, -/+ S9 (based on preliminary toxicity tests): 0, 0.25, 0.5 & 1 mg/ml; positive controls: -S9, 1 mg/ml N-methyl-N'-nitro-N-nitrosoguanidine; +S9, 0.5 mg/ml dimethylnitrosamine; exposure time: -S9, 24 & 48 hr; +S9, 4 hr; 4 x 103 cells/flask seeded (flask size not given), duplicate cultures exposed/condition, test article exposure began 48 hr later; colchicine added 2 hr prior to harvest to arrest cells in metaphase; 50 metaphases examined/flask (100/condition total); possible slight increase in % cells with chromosome aberrations under -S9 condition (control cells @ 48 hr w/aberrations excluding gaps = 1%, exposed cells = 2, 5, & 4%, respectively), but beneath the 10% limit considered by the investigators to be biologically relevant; no increase in +S9 cells; positive controls were successful; **Unacceptable, but may be upgradeable** upon submission of test article purity and size of flask used in assay. (Rubin,

4/29/93)

51950-025; 119531; 844; other genotoxic effects; "Unscheduled DNA Synthesis [UDS] in Primary Hepatocytes of Male Rats In Vitro with WAK 3839"; author: R. Fautz; 4/24/89; report #100665; 98.9% purity; hepatocytes (derived freshly from male animals because female-derived cells purportedly lack certain activating enzymes) seeded at 105/ml in 35 mm culture dishes containing 1-25 mm cover slip; doses (based on a preliminary cytotoxicity determination and test article solubility), Expt. I & II: .04, .13, .44, 1.33, 4.44, 13.33, 44.44, 133.33, 444.44, & 1333.33 mg/ml (last 2 doses Expt. II only); Expt. III: 13.33, 44.44, 133.33, 444.44, & 1333.33 mg/ml; 18 hr exposure; triplicate dishes run at each concentration; positive control: 11.16 mg/ml 2-acetyl aminofluorene; UDS measured by autoradiographic determination of 3H-thymidine incorporation into DNA; severe cytotoxicity observed only in Expt. I above 133.33 mg/ml (other expts. were negative); no reproducible test article dependent increase in incorporation was observed under any condition despite the consistent success of the positive control; test article does not induce UDS in this system under the conditions tested; **Unacceptable, but possibly upgradeable** with submission of cytotoxicity data. (Rubin, 4/29/93)